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ANALYSIS OF CLAISEN-SCHMIDT CONDENSATION PRODUCTS BETWEEN 2-HYDROXY-ACETOPHENONE AND P-ANISALDEHYDE IN BASIC MEDIUM

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Abstract: The search for biologically active substances is of great importance, mainly due to the fact of bacterial resistance against the existing therapeutic arsenal. Flavonoids, substances from secondary metabolism, can be a good alternative. Due to these factors, this work reports the study of the reaction of 2-hydroxy-acetophenone and p-anisaldehyde, in an attempt to obtain 2'-hydroxy-chalcone, an intermediate in the synthesis of flavonoids. In this study, the products formed by varying the reaction conditions were analyzed, under reflux at 90°C for 3 hours and 10% NaOH solution; at room temperature, under stirring and without heating with 10% NaOH; and for 3 hours of magnetic stirring using a 50% NaOH solution. Analyzes were performed by HPLC, Mass Spectrometry and Hydrogen RMN.
Keywords: Condensation Reaction, Chalcone, Flavanone.

INTRODUCTION

The search for substances with biological activities for use in the pharmaceutical industry is basically done in two ways. One is the structural modification of existing drugs, which has proven to be an effective means of extending the shelf life of several drug groups. The other is the development of new classes of drugs that can act on different biological targets, such as natural products, flavonoids, alkaloids, acetogenins, in addition to synthetic substances.

Flavonoids, which are derived from secondary metabolism, constitute a family of diverse molecules, containing two aromatic rings, which are connected by a pyran ring. These compounds can be grouped into the following main subgroups: chalcones, dihydrochalcones, flavones, flavonols, catechins or flavanols, flavanones, anthocyanins and isoflavones (Figure 1) (HOFFMANN-RIBANI and RODRIGUEZ-

AMAYA, 2008).

To be one of the promising classes, as the literature reports antimicrobial activities via inhibition of nucleic acid synthesis, inhibition of protein synthesis, inhibition of lipid synthesis and inhibition of plasma membrane functions, they also have antioxidant activity (FLORIANO et al., 2020), antiviral and antibacterial activity (WANG; LI; BI, 2017), antifungal activities, inhibiting the germination of spores of plant pathogens, antiviral activities, against the human immunodeficiency virus (HIV) (CUSHNIE and LAMB, 2005) and activity anti-inflammatory (KIM et al., 2004), among others.

Chalcones, represented by structure I, belong to the class of flavonoids (DEWICK, 2001) and are considered as essential intermediaries in the biosynthetic route of flavonoids (DIXON and STEELE, 1999), which are formed by the cyclization of the hydroxyl located in the 2` position of the chalcones (Figure 2) (NAKANISHI, 1975).

2'-hydroxy-chalcone is the main intermediate for obtaining flavonoids, and can be obtained by the reaction between 2-hydroxy-acetophenone and substituted benzaldehydes(MPHAHLELE and FERNANDES, 2002), however, several other products can be obtained (Figure 3), which decreases the yield of obtaining the chalcone of interest.

With the objective of obtaining more favorable conditions for obtaining 2'-hydroxy-chalcones, which will be used in the synthesis of flavonoids, three experiments were carried out, varying the reaction time, catalyst concentration and reaction temperature, using 2 -hydroxyacetophenone and p-anisaldehyde.

METHODOLOGY

EQUIPMENT USED

Waters Acquity TQD mass spectrometer, Bruker Ascend 400 NMR spectrometer (400 MHz), Alliance e2695 line chromatograph (Waters) (Postgraduate in Chemistry/UFPA).

REAGENTS AND SOLUTIONS

The reagents used were Aldrich, Vetec or Nuclear, all PA.

HPLC/DAD CHROMATOGRAPHIC PROCEDURE

The development of the chromatographic method for analysis of the reaction products was carried out in an Alliance e2695 line chromatograph (Waters), with a binary pump system and automatic injector coupled to a UV/Vis detector with diode arrangement covering the length range of 210 – 600 nm wave. The stationary phase was a Sunfire C18 reversed phase column (150 x 4.6 mm, 5μm), with a Sunfire C18 guard column (20 mm x 4.6 mm, 0.5 μm) and flow rate of 1mL/min in an oven thermostatic at 40ºC. The sample was eluted in HPLC grade methanol (Jt Baker®) and subsequently filtered through nylon filtering membranes with pores of 0.45 μm, from the Millipore brand (Tullagreen, Carrigtwohill, Ireland. The mobile phase consisted of a binary mixture of ultra pure water (GEHAKA) and MeOH filtered in a linear exploratory gradient of elution in the method proportion of H2O-MeOH 90:10 to 0:100 of B in 60 min. Samples were injected with a volume of 20 μL.

REACTION PROCEDURES

Procedure A:

In a 250mL erlenmeyer flask, 20mL of ethanol, 3g of 2-hydroxy-acetophenone, 10mL of sodium hydroxide solution (10%) were added and kept under magnetic stirring at room temperature, then 3mL of p-anisaldehyde were added. This mixture was kept under agitation (Ta) for about 3 hours, after which it was stored in a freezer for 24

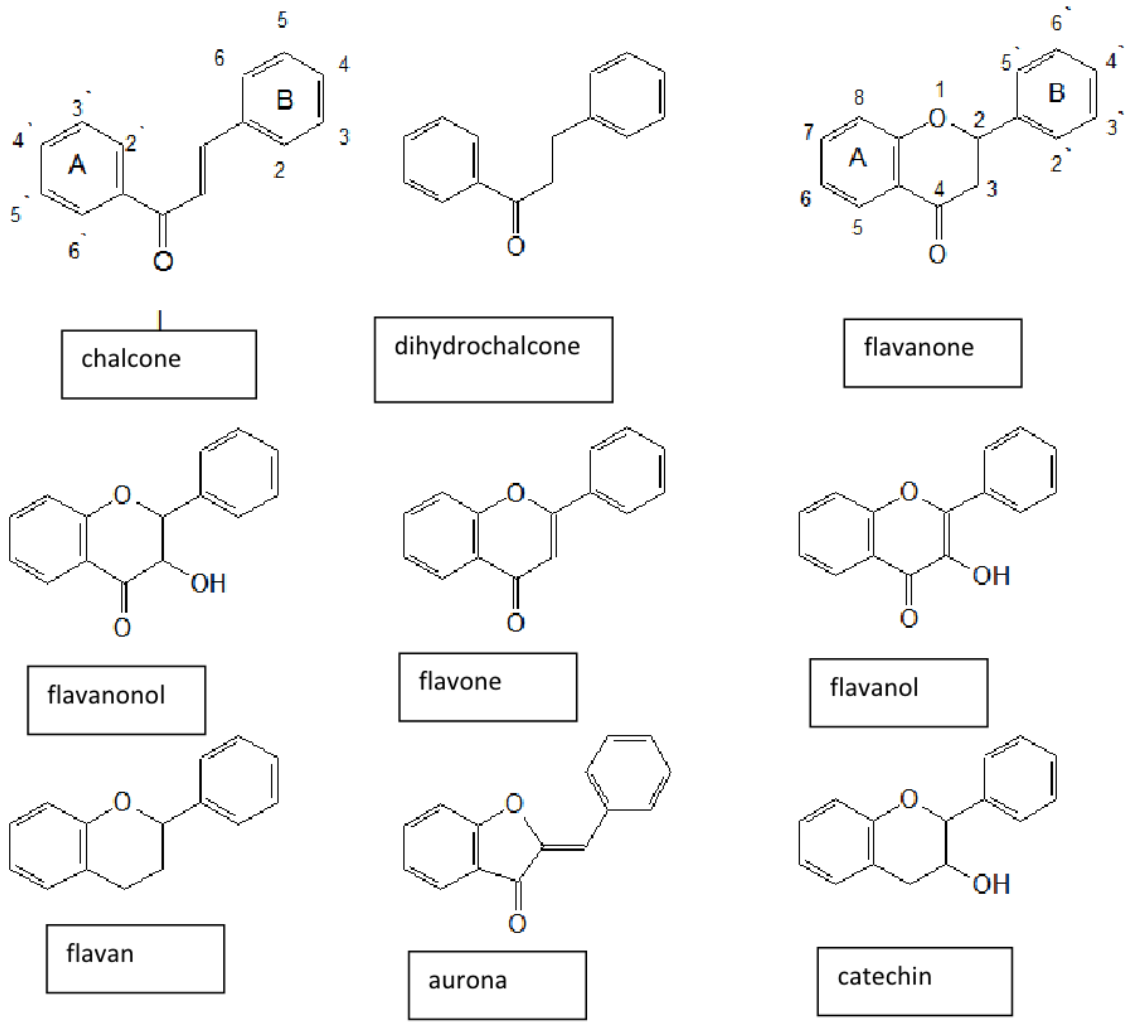

Figure 1 – General chemical structure of the flavonoid class.

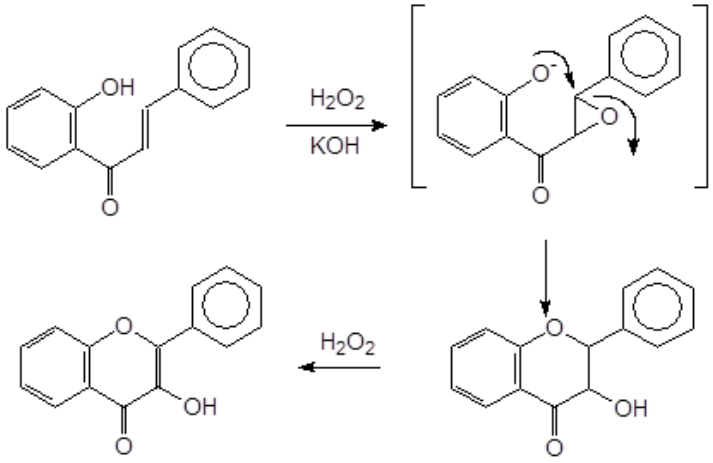

Figure 2 – Scheme of the chemical equation for the synthesis of Flavonoids.

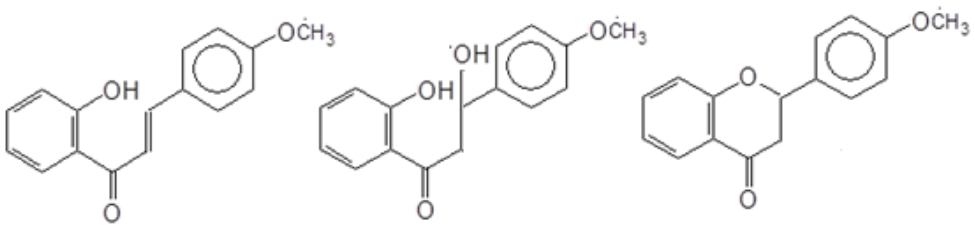

Figure 3 – Probable reaction products between 2-hydroxy-acetophenone and p-anisaldehyde.

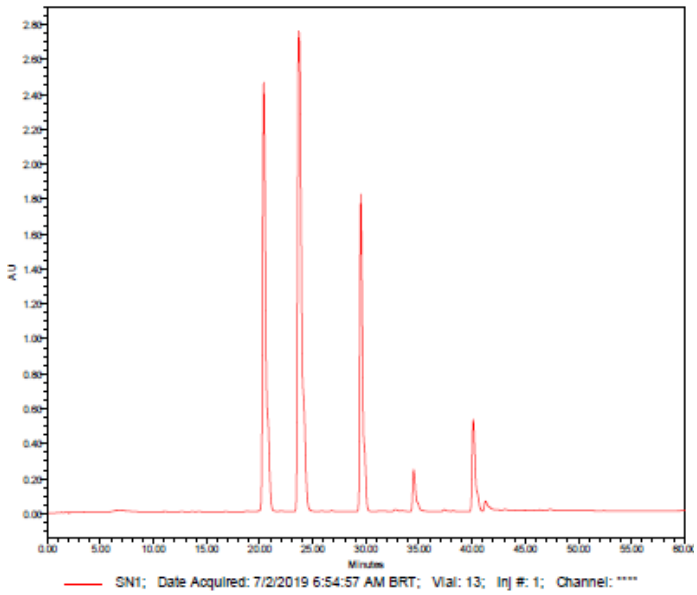

Figure 4 – Chromatogram of procedure C.

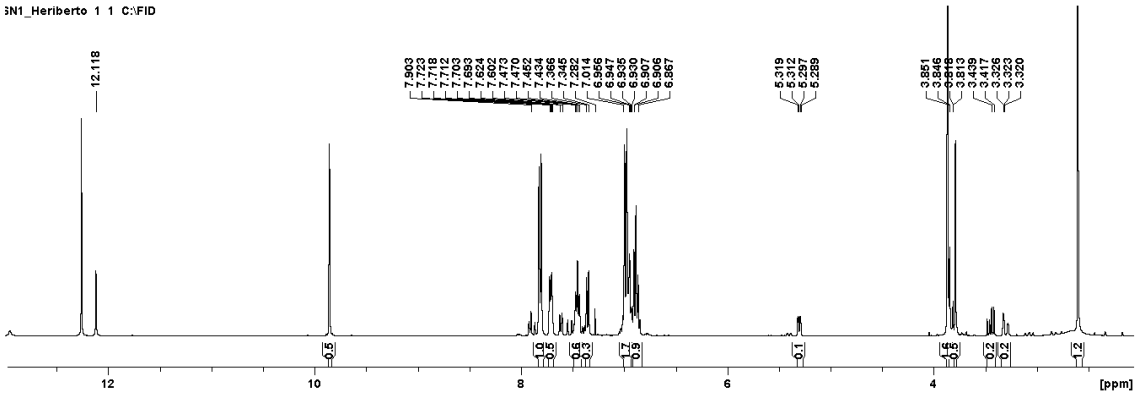

Figure 5 – 1H-NMR spectrum (400MHz; CDCl3) of procedure C.

hours. After this time, it was acidified with acetic acid solution (20%) to an acidic pH. The acid solution was subsequently subjected to extraction with CH2Cl2 PA in a 250 mL decantation funnel, three times, to obtain the dichloromethane solution. This solution was washed with distilled water (3 times) and dried with anhydrous Na2SO4, filtered and evaporated, providing the products quantitatively.

Procedure B:

In a 250mL erlenmeyer flask, 20mL of ethanol, 3g of 2-hydroxy-acetophenone, 10mL of sodium hydroxide solution (50%) were added and kept under magnetic stirring at room temperature, then 3mL of p-anisaldehyde were added. This mixture was kept under agitation (Ta) for about 3 hours, after which it was stored in a freezer for 24 hours. After this time, it was acidified with acetic acid solution (20%) to an acidic pH. The acid solution was subsequently subjected to extraction with CH2Cl2PA in a 250 mL decantation funnel, three times, to obtain the dichloromethane solution. This solution was washed with distilled water (3 times) and dried with anhydrous Na2SO4, filtered and evaporated, providing the products quantitatively.

Procedure C:

In a 250mL flat-bottomed flask, 20mL of ethanol, 3g of 2-hydroxy-acetophenone, 10mL of sodium hydroxide solution (10%) and then 3mL of p-anisaldehyde were added under magnetic stirring. A straight tube condenser was attached to the flask with the reaction mixture and kept under reflux (temperature of 90°C) with magnetic stirring for about 4 hours, after which time it was stored in a freezer for 24 hours. After this time, the solution was acidified with acetic acid (20%) to an acidic pH. The acid solution was subsequently subjected to extraction with CH2Cl2 PA in a 250 mL decantation funnel, three times, to obtain the dichloromethane solution. This solution was washed with distilled water (3 times) and dried with anhydrous Na2SO4, filtered and evaporated, providing the products quantitatively.

RESULTS AND DISCUSSION

By analyzing the chromatograms of the reactions, it is verified that in procedures A and B, there are 3 peaks, while in the chromatogram of procedure C (Figure 4), there are 5 peaks, 2 more. Being two with different retention time. In relation to the 3 peaks of A and B. Also, it appears that the third peak is found with greater intensity in B.

The analysis of the Hydrogen NMR spectrum of procedure C (Figure 5), indicates the absence of 2'-hydroxy-chalcone, the necessary intermediate for the synthesis of flavonoids, due to the absence of the doublet with coupling of ~16Hz, characteristic of these substances, because they have a trans configuration in the double bond. However, signals are observed for methyl hydrogens, at δ2.68ppm (probably from the starting material; 2-hydroxy-acetophenone), relative signal for aldehyde hydrogen δ12.1ppm (probably from the starting material; para-anisaldehyde) and signals relative to the hydrogens of the pyran ring at δ5.30 (dd; Jtrans= 9Hz and Jcis= 3Hz), δ3.45 (dd; Jgem=17Hz and Jtrans=9Hz) and δ3.30 (dd; Jgem=17Hz and Jcis=3Hz), probably due to the formation of flavanone (HARBONE, 1975).

The analysis of the mass spectrum of procedure C, indicated the presence of a load/mass peak of 254 (100%) and 238 (83%), corresponding to flavanone (C16H14O3); corresponding charge/mass peak 136 of 2'-hydroxy-acetophenone and para-anisaldehyde (C8H8O2) and charge/mass peak at 272 (5%) corresponding to the reaction

intermediate, probably 1-(2'-hydroxy-phenyl) -3-hydroxy-3-(4-methoxy-phenyl)-propan-1-one (C16H16O4). Besides, no information regarding 2'-hydroxy-chalcone was observed, confirming the information obtained via the 1H-NMR spectrum.

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